Reference: *Biol. Bull.*, **145**: 340–351. (October, 1973)

REPRODUCTION TESTS: THE TOXICITY FOR *ARTEMIA* OF DERIVATIVES FROM NON-PERSISTENT PESTICIDES[1]

DANIEL S. GROSCH

Genetics Department, North Carolina State University, Raleigh, North Carolina 27607; and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

The primary objective of the present study was to investigate the reproductive performance of a small crustacean after exposure to sublethal doses of the types of organic compounds occurring early in the degradation of non-persistent pesticides. In choosing the compounds to be tested, our guide was the United States Department of Interior monograph by Menzie (1969). Although the original toxicant may be converted within days or weeks, aromatic ring structures can persist. One transformation well established for sea-water is the hydrolysis of Sevin® to 1-naphthol (Stewart, Millemann and Breese, 1967). A more comprehensive study of the steps of biological degradation in the aquatic environment has employed river water and sewage (Aly and El-Dib, 1972).

Using the brine shrimp *Artemia salina* Leach as the assay organism, we conducted an exploratory screening experiment on nine different compounds. Three of these failed to reduce adult life span or depress fecundity at 10 parts per million (ppm): benzazimide and 3-hydroxy-methylbenzazimide from azinphosmethyl, and 3,5,6-tricholoro-2-pyridinol from Dursban®. This paper gives the results of a follow-up study of the 6 remaining agents listed on Table I.

Complete reproductive records are summarized for adult *Artemia* of two different histories, (1) those removed to standard rearing conditions after only one day's exposure, and (2) those maturing a year later in treated mass populations reconstituted after routine over winter evaporation. Observations on the mass populations are also presented.

Materials and Methods

The stock of *Artemia* used for the present experiments was begun in 1957 from a mass hatching of thousands of California cysts. By 1971 when these experiments began, the population had 14 years of adaptation to laboratory conditions. Characterization of the reproductive performance of the stock is given in previous papers (Grosch, 1966, 1967). Since its origin the stock has over-wintered on shelves in windows with South West exposure in rooms maintained above 50° C at the Marine Biological Laboratory of Woods Hole, Massachusetts. Most important for the successful mass rearing of *Artemia* is my use of annual evaporation to eliminate the repressive influence of accumulated secretions and excretions. This occurs during the winter months. Annually in June cultures are reconstituted by adding

[1] Supported by PHS Grant ES-00044, National Institute of Environmental Health Sciences. Published as a short scientific report with the approval of the Director of Research, North Carolina Experiment Station. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

TABLE I

The tested compounds listed in relation to the pesticides from which they can be derived

Name of pesticide	Chemical name	Derivative tested	Abbreviation used
Parathion	0,0-diethyl 0-(p-nitrophenyl) phosphorothioate	p-nitrophenol	p-N
Diazinon	0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	2-isopropyl-4-methyl-6-hydroxy-pyrimidine	Iso
Carbaryl (Sevin)	1-naphthyl-N-methylcarbamate	1-naphthol	1-N
		1,5-dihydroxy naphthalene	1,5-D
		1-naphthyl-hydroxy-methyl carbamate	C
Trichlorobenzene*	1,3,5-trichlorobenzene	1,3,5-trichloro-benzene	TCB

* Chlorinated benzene compounds have been used for termite control and also are derived in the degradation of several types of pesticides.

distilled water to the salts and cysts deposited during the gradual evaporation. Under these conditions, a 20-liter battery jar will show more than 2000 larvae and produce 1000 adults.

In 1971 when post-nauplial larvae had developed, 1-liter portions of the mass cultures were distributed to a number of subculture jars which were gradually brought up to 3 liters by small daily additions of filtered sea-water. As they matured, shrimp were distributed to provide 100 adults in each 3-liter subculture. Each compound tested was dissolved in 1 ml of acetone and stirred into one of the subcultures to provide a simple ecological system of volunteer algae, shrimp, and sea-water containing 10 ppm of the chemical. In our experience with *Artemia* (Grosch, 1967) as well as in general toxicology, acetone has proved to be relatively harmless (Moeschlin, 1965) and useful as an organic solvent.

In the preliminary screening, exposures up to 4 days were attempted and the solutions tested ranged downward from 100 ppm to 0.01 ppm. Above 10 ppm not all compounds were soluble. With those that were, deaths resulted even after short exposures. Exposures at 10 ppm for 2 days appeared to approach the early-death limit for several agents. On the other hand, decreases in reproductive performance were not always evident after exposures to 1 ppm. A compromise was to remove one group of adults for study after a day's exposure to 10 ppm, continue observation of the treated mass culture through the summer of 1971, and a year later study reproductive performance of a sample of adults from each population still viable.

Therefore, 24 hours after addition of a test compound, ten pairs were taken from each treated population. Each mated pair was placed in a separate jar containing

TABLE II

Adult life span and the components of their reproductive performance summarized as averages for samples of Artemia *comprised of 10 pairs subjected to each treatment of 10 ppm for 24 hours in 1971. Standard errors were calculated for all means but are not listed in some categories to conserve space. A basis for recognizing 2 classes is given in the text*

Chemical tested	Survival of adults (days)		Number of broods	Total no. of zygotes	Cysts (%)	Cysts hatched (%)	Larval survival (%)	Sex ratio no. males/ no. females	Adaptive value
	Males	Females							
None	49.6 ± 4.0	50.0 ± 5.0	11.3 ± 1.4	1828	29.0	46 ± 5	76.3 ± 5.0	0.91	
Acetone	47.6 ± 4.2	50.1 ± 5.5	11.8 ± 1.6	1884	30.6	48 ± 7	75.6 ± 4.7	0.94	1.00
Class I									
p-nitrophenol	37.7 ± 5.4	34.8 ± 5.1	5.7 ± 1.8	1031	33.8	44 ± 6	72.4 ± 5.6	0.86	0.48
2-isopropyl-4-methyl-6-hydroxy pyrimidine	37.3 ± 2.7	33.6 ± 2.1	8.2 ± 0.8	1060	3.7	61 ± 9	70.2 ± 8.6	0.79	0.61
1-naphthol	30.9 ± 3.4	31.3 ± 2.4	6.3 ± 0.9	1062	11.6	50 ± 11	84.6 ± 6.8	0.97	0.70
Class II									
1,5-dihydroxy naphthalene	25.2 ± 2.5	22.1 ± 1.9	4.9 ± 0.7	463	21.5	42 ± 8	46.2 ± 12.9	0.95	0.16
1-naphthyl hydroxymethyl carbamate	23.3 ± 9.6	33.6 ± 9.2	7.2 ± 1.9	694	2.8	80 ± 12	52.6 ± 7.9	0.54	0.38
1,3,5-trichloro-benzene	44.2 ± 3.8	37.6 ± 4.2	5.3 ± 0.8	456	11.4	18 ± 10	30.3 ± 11.5	0.82	0.11

500 ml of a standard brine solution (Grosch, 1967). Subsequently each pair was fed 0.5 ml of yeast suspension daily. Before each feeding, zygotes were removed and counted. Larvae produced viviparously were transferred by groups into separate rearing jars, maintained until maturity in standard brine. Cysts were filtered, dried and resuspended in sea-water to determine the proportion from which larvae could emerge. Both the pair-mating jars and the rearing jars were kept under constant fluorescent light which holds the water temperature between 26°–28° C.

After 10 pairs had been removed for life-time reproductive performance studies, the 3-liter treatment jars with 80 adults were shelved. Jars showing live *Artemia* were fed daily with yeast suspension. In all jars the water level was maintained until September 1 when the annual evaporation phase began.

In early June of 1972 cultures in the treatment jars were reconstituted by adding distilled water to their contents. In jars where adults matured, again pairs were set up in individual jars of brine to obtain data on life span and reproductive performance.

When summarized data were tested statistically, t values were calculated from the ratio of the mean difference to the variance of the difference. Upon comparison with the standard *t* table the 0.05 probability level was taken as significant and the 0.01 level as highly significant evidence against the null hypothesis.

A mathematical approach to fitness which will be shown of limited predictive utility for *Artemia* populations is the "adaptive value." In its simplest form, this

TABLE III

Adult life span and the components of their reproductive performance summarized as averages for samples of the Artemia *adults matured in the mass populations reconstituted in 1972 from an overwintering evaporation phase. Standard errors were calculated for all means but are not listed in some categories to conserve space*

Chemical tested	1972 Generation tested	Adult life span (days)		Number of broods	Total no. of zygotes	Cysts (%)	Cysts hatched (%)	Larval survival (%)	Sex ratio no. males/ no. females	Adaptive value
		Males	Females							
Acetone control	1st	41.1 ± 4.3	40.0 ± 4.9	10.0 ± 1.4	1221	31.1	58 ± 5	70.2 ± 6.1	0.96	1.00
p-nitrophenol	1st	33.3 ± 3.5	30.5 ± 4.0	6.3 ± 1.0	1239	33.1	59 ± 4	65.2 ± 8.4	0.96	0.93
2-isopropyl-4-methyl-6-hydroxy pyrimidine	1st*	12.0	24.0	4.0	365	86.8	67 ± 8	75.2 ± 8.7	0.82	0.26
	2nd	36.0 ± 2.2	32.4 ± 1.0	8.0 ± 0.8	1148	15.4	60 ± 4	68.2 ± 7.7	0.99	0.97
1,5-dihydroxy naphthalene	1st	26.3 ± 4.6	21.6 ± 1.7	3.1 ± 0.4	523	70.2	76 ± 3	64.1 ± 6.6	0.92	0.37

* The initial 1972 generation consisted of 3 adult females and 1 male. All other data tabulated come from 10 pairs from each population.

measurement consists of dividing the average number of matured offspring per pair by the average number of matured offspring per control pair. Often for simple comparisons one of the strains is arbitrarily designated as having a fitness of 1.00 (Strickberger, 1968). For our purposes the acetone control was so designated. The adaptive values obtained (A.V.) from pair mating tests are given on Tables II and III and evaluated in the Discussion.

RESULTS

1971 pair mating tests

These results are summarized in Table II. A trend toward decreased life span was revealed for all treated adults. While a statistically significant difference from the control mean cannot be established for males exposed to 1,3,5-trichlorobenzene "TCB," the premise of shortened life spans can be accepted for all other treated groups. When the difference between means is 12 days, t values achieve the 0.05 level; when the difference is 15 or more days, t exceeds the 0.01 level. In general, the incidence of death was distributed so equally between the sexes that when a male died his female could be supplied from the same test group with a male that had recently lost his mate. Assuring females of mates was possible even in the 1-naphthyl hydroxymethyl carbamate "C" tests when males tended to die earlier than females.

The average total number of broods per pair was decreased by every agent. Statistically when the difference from the control mean amounts to 5 or more broods, t values exceed that for $t_{0.995}$. Even when the difference is only 3.1 (11.3–8.2) t approached the 0.5 level.

In most of the experimental groups the deficit could be a consequence of the decrease in life span. With successive broods appearing at 3 to 4 day intervals, shortening the female life span by 15 days results in 4 or 5 broods fewer than the control pairs. In addition, 1,5-dihydroxynaphthalene "1,5-D" and TCB caused a delay in the appearance of the first brood for more than a week. Thus, an addi-

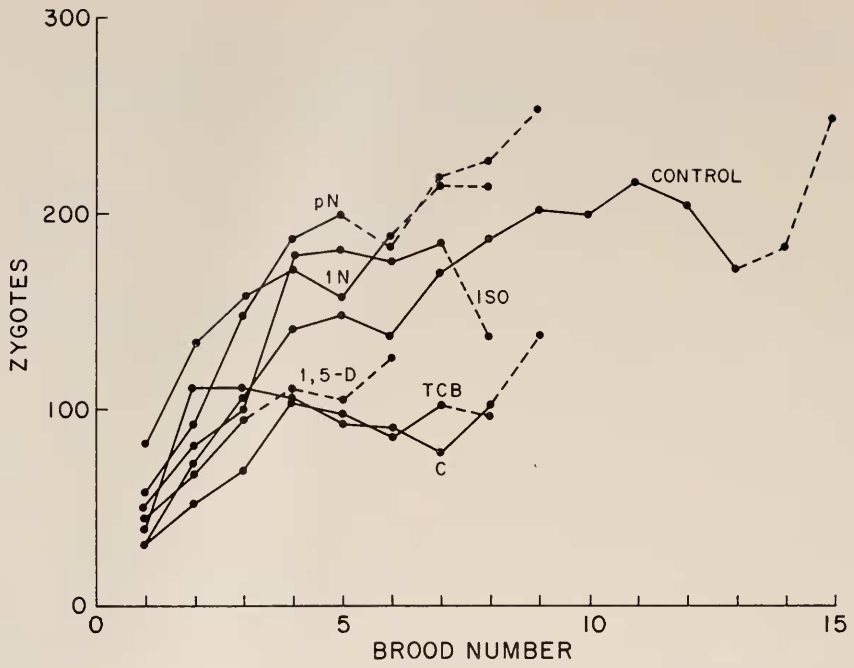

FIGURE 1. The average brood size plotted in the sequence of its production by pairs withdrawn after 24 hours from the populations of *Artemia* subjected to degradation products of pesticides in 1971. Abbreviations are: pN, p-nitrophenol; 1 N, 1 naphthol; ISO, 2-isopropyl-4-methyl-6-hydroxypyrimidine; 1,5-D, 1-5-dihydroxynaphthalene; C, 1-naphthyl hydroxymethyl carbamate; TCB, 1.3,5-trichlorobenzene. The broken line indicates that less half of the pairs remain alive.

tional two or more broods were subtracted from a potential life time total curtailed by a shortened life span. During the sterile periods there was no reluctance to mate. Direct observations of pair activities ruled out a change in mating behavior.

A decrease in the number of broods was accompanied by a decrease of the average total number of zygotes produced. Such an influence explains the three deficiencies of about 800 zygotes giving totals of slightly more than 1000 zygotes, but it does not explain the other three cases where life time totals amounted to less than a third of the control value. Plotting the average size of each brood in the sequence of its production (Fig. 1) reveals two classes of response to the chemicals tested. In one class three of the experimental groups produced offspring at a rate equal to or exceeding that of the controls but they were not living long enough to produce a series of broods in the 200 offspring category attained by the controls. On the other hand, the 3 poorly performing groups of Table II are shown in Figure 1 to have had a trend to brood size smaller than controls. This trend becomes evident by the third brood. Thus, in a second class of experimental shrimp certain chemicals decreased the number of zygotes per brood. When averages are calculated the three groups of the second class averaged under 100 zygotes per brood, respectively 94.5, 96.4 and 86.0 zygotes per brood.

Further damage from the three more deleterious compounds was revealed in the lower larval survival (Table II), a result especially notable in *Artemia* where relaxation of crowding is ordinarily accompanied by improved survival to maturity. In contrast, the offspring from controls and from the three less deleterious experimental groups showed an excellent proportion of larvae surviving to maturity. A predominance of the digametic sex (females) among matured larvae, shown by sex ratios below 1.00, rules out the action of significant numbers of induced recessive sex-linked lethal mutations.

In the tests summarized in Table II the treated females produced too few cysts to provide reliable evidence on cyst hatchability. In particular the "C" test provided almost no cysts and a decrease in oviparity was noted for 4 of the other 5 experimental groups.

Treated populations in 3-liter jars

Three of the populations survived less than a week because all the adults remaining in the mass rearing jars died without producing young of either type. These were the jars receiving either 1-naphthol "1-N," 1-naphthyl-hydroxymethyl-carbamate "C," or 1,3,5-trichlorobenzene "TCB." Although cysts were recovered from the 1-N jar, none contained viable embryos. In the other three treated populations adults remained alive for the entire summer. Despite recurrent pairing and copulation, viviparity was rare, and post-naupliar stages never seen. Cysts accumulated at the high water line only during the first few days.

Despite the water's persistent intense yellow color, mature adults of the p-nitrophenol "p-N" culture were able to survive the entire summer of 1971. At reconstitution of the evaporated culture in June, 1972, many yellow crystals were present among the salts at the bottom of the jar. When the salts dissolved, the brine again had a definite yellow color. Nevertheless there was a massive hatch of cysts from the sides of the jar, and more than 100 adults matured. In turn these produced viviparous young during 1972. Several hundred shrimp from these broods survived to maturity.

Upon rehydration in 1972 of the culture given 2-isopropyl-4-methyl-6-hydroxy-pyrimidine "Iso" there was a copious hatching of cysts but only 4 females and 1 male survived to maturity. After producing a first brood of larvae, these 5 adults were removed from the mass culture jar for a study of their reproductive performance. By July 20, 1972 the larvae remaining in the 3-liter jar had given rise to more than 100 adults. Presumably removal of the adults from the jar expedited maturation of a second summer generation. Of these, the reproductive performance of 10 pair matings was studied in individual 500 ml jars.

After receiving 1,5-dihydroxynaphthalene "1,5-D" the 3-liter culture developed a dirty yellow cloudiness during the first 24 hours and an odor of naphthalene which persisted for over a week. Subsequently finely divided black debris settled out of the culture in the course of the summer. Some of this was identifiable as dead larvae. At culture reactivation in 1972 numerous larvae hatched from the cyst layer at the high water mark. Although survival to maturity seemed poor, more than 100 reproducing adults comprised the population by the end of August.

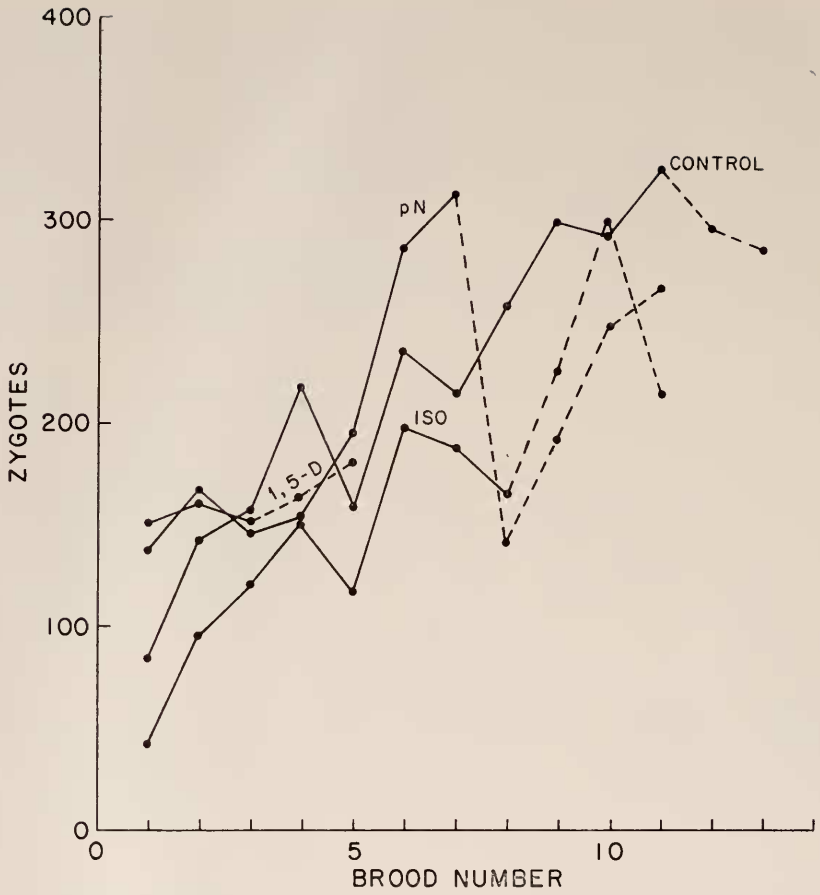

FIGURE 2. The average brood size plotted in the sequence of its production by pairs from the populations of *Artemia* surviving the evaporation-reconstitution cycle of 1971 to 1972. Abbreviations are: pN, p-nitrophenol; ISO, 2-isopropyl-4-methyl-6-hydroxypyrimidine; 1,5-D,1,5-dihydroxynaphthalene. The broken line indicates that less than half of the pairs remain alive.

1972 pair mating tests

Results are summarized in Table III for the jar populations that survived to provide shrimp for testing a year after treatment. Adults from treated populations tended to die earlier than those from the control, but in 1972 the mean life span of controls was about a week shorter than in 1971. Accordingly, the differences between means are not large, and t tests reveal significant decreases only for males and females of the 1,5-D group. The first IMH generation of only 1 male and 3 females was inadequate for such analysis.

A ranking of the average number of broods is concomitant with a ranking of female life spans. The t value for 1,5-D exceeds that for $t_{0.995}$. Even the t value

for p-N number of broods exceeds the 0.05 level. On the other hand, the p-N pairs had an average of total zygotes higher than that of the year's controls.

An appreciable proportion of zygotes were encysted for all groups tested, and the average percentage hatching exceeded the control value in all experimental groups. In 1972 the survival of larvae to maturity approached the control level in data from pair tests. The relaxation of crowding in the few broods from the 3 females available as the first 1972 generation of the IMH treated population is reflected in the 75.2% average survival of larvae. This exceeds the control value but not to a degree considered statistically significant. The sex ratios among the maturing offspring were all under 1.00 but consistently high. For all groups tested, first broods appeared without delay, and subsequently, broods were deposited every 3 to 4 days with regularity.

Plotting the average size of each brood in the sequence of its production (Fig. 2) reveals that pairs from the p-N populations exceeded the 200 offspring per brood level after the fifth brood. This was accomplished by a higher rate of increase in brood size than that of controls from the 4th to 7th broods deposited. On the other hand, in comparison with the controls, smaller broods were produced by the females of the Iso treated population, although the increase in brood size occurred at about the same rate as the controls. In contrast, the size of the broods deposited by females from the 1,5-D population was adequate for early broods. However, most of the shrimp from this population died after producing only 3 broods.

Discussion

The pesticides of concern may enter coastal waters not only from their use in forestry and agriculture, but also through attempts to control estuarine shellfish predators (Karinen, Lamberton, Stewart and Terriere, 1967). An incentive for this research was the disquieting thought that the initial actions of environmental degradation may not provide biologically inert products from the molecules of so-called non-persistent pesticides. In addition, one category of derivatives, the naphthalene compounds, can escape to the environment from the manufacture of dyes, synthetic resins, explosives, lubricants, and motor fuels.

A wide variety of effects from pesticides on non-target organisms has been reviewed by Pimentel (1971), but with crustacea attention has tended to focus on the determination of immobilization and lethal doses. A notable contribution to crustacean toxicology has been the development of the *Daphnia magna* bioassay (Parker, 1965; Frear and Boyd, 1967). However, here too, emphasis has not been given to reproductive performance. A special feature of the present study is the determination of the number and fate of *all* zygotes in the *sequence* of their production. The mammalian design of the reproduction test which employs one selected litter or a group of several litters (Fitzhugh, 1968) is inadequate for an organism like the shrimp where size is indeterminate and brood size increases with the increasing size and age of the mother. Within this framework it seems important to distinguish between toxic agents that may merely cause maternal debility (Class I) and those which include a more direct attack upon reproduction (Class II). Postulated to explain the Class II data of Table II is the vulnerability of dividing cells. On this basis, damage is expected in the stem cell component of the *Artemia* gonads and to the cells of the cleaving embryo. Embryos attain the blastula stage

within the female before a choice between encystment or viviparity is made for them (Lochhead, 1941).

In mammalian toxicology, deficiencies in proliferating cell populations are caused by compounds chemically related to those of our Class II. Benzene and its derivatives including naphthalene are known to cause bone marrow depletion with associated leukopenia and anemia (Moeschlin, 1965). Less familiar are the cytological investigations with plant meristems which demonstrated that members of the halogenated naphthalene series inhibit mitosis in low doses and are cytotoxic at higher concentrations (Gavaudan and Gavaudan, 1940; Levan and Ostergren, 1943). However, until now none of the naphthalene and carbamate compounds of Table I seem to have received cytogenetic consideration. Most recently we have obtained data on the fecundity and fertility of *Habrobracon* (Hymenoptera: Braconidae) females following exposure to the three most effective compounds (Grosch and Hoffman, 1973). In this wasp the single series of oogenetic stages per ovariole make possible an exact identification of the most sensitive types of cells. These proved to be the oogonia engaged in the five mitotic divisions necessary to form the folliculate nests, each containing an oocyte and 31 nurse cells (Grosch, 1965). Cells irrevocably damaged at this point never form oocytes. Others give rise to oocytes but their embryos fail to complete the nuclear cleavage divisions. These braconid results are strikingly parallel to those for *Artemia*, but a complete correlation of ovarian cell responses between the two quite different Arthropods will require experiments in which only female *Artemia* are exposed to the naphthalene compounds. On the other hand, there is a definite difference in somatic sensitivity between the adult insect and the adult shrimp. The braconid is relatively tolerant because its somatic tissues are no longer mitotic, but the female shrimp molts before each brood is produced (Lochhead, 1941). Since adult males also continue to molt and grow throughout adulthood, the presence of cell divisions may be presumed. Thus, epidermal mitoses associated with the periodic molting of both sexes may help to explain the particularly short adult life spans following exposure to naphthalene compounds.

The biodegradable pesticides providing the derivatives tested have received more attention. In chickens, ingested carbaryl decreased egg hatchability and induced congenital malformations (Ghadiri *et al.*, 1967). However, at doses 1000 times the allowable level of the pesticides, carbaryl produced terata only in guinea pigs, not in hamsters and rats (Robens, 1969). Diazinon was non-teratogenic in small laboratory animals (Robens, 1969). In a 3 generation reproductive study (Collins, Hanson and Keeler, 1971), rat and gerbil fertility was impaired by 10,000 ppm of carbaryl (Sevin®) in the food, but a more recent summary of all the investigations using dietary doses no higher than 2000 ppm concluded that there are no reproductive effects in rodents at realistic dose levels (Weil, Woodside, Carpenter, and Smyth, 1972). In mammals and insects carbamate and naphthalene molecules are metabolized by the microsomal enzymes (Parke, 1968). A survey of marine forms showed Sevin more toxic to larval and adult crustaceans than to molluscs and fish (Stewart, Millemann and Breese, 1967), but no data was obtained on reproductive performance. Subsequently flaccid ovaries were found to be in a resorptive state for the infecund minnows resulting from the highest dose level of a series of 9 month exposures to Sevin (Carlson, 1972).

Some of the consequences of exposing shrimp populations to carbamate derivatives is provided by observations reported here. Generally, fitness is defined as the relative capacity for leaving offspring that attain reproductive age (Mettler and Gregg, 1969). To accomplish this, *Artemia* along with many other organisms have adopted the reproductive strategy of investing their energy in gamete production. The loss of a considerable proportion of potential offspring can be serious. A reduction of the number of zygotes to $\frac{1}{3}$ or less than the control average, as caused by 3 of the tested compounds, implies impending population collapse. In long term studies of irradiated populations, more than half of the normal reproductive capacity constituted a reserve necessary for buffering environmental changes (Grosch, 1966).

In addition to quantity, the quality or type of zygote is significant for *Artemia* population survival. Shunting offspring into a dormant encysted state is the usual *Artemia* response to unfavorable external factors. By interfering with this response most of the chemicals added to jar cultures in 1971 had the effect of increasing the vulnerability of shrimp to their environment. Furthermore, response of the shrimp could not be predicted from the appearance of the water in which they lived. The two visibly contaminated cultures have survived while others with clear water became exinct.

The proportion of zygotes encysted may be the most important index to strain survival, provided some of the embryos are viable. Since cyst production is a prompt response important for surviving an environmental change, it was not surprising to observe a significant shedding of cysts within a day or two after a chemical contamination of a jar population. At the time this important component of cysts was produced, the adults of the entire population were equivalent to those removed for pair mating tests. Subsequently, the reproductive capacities of the mass population and the pairs removed to uncontaminated water diverged.

Although the 1971 A.V.'s serve as a basis for comparing the reproductive performance of pairs removed from the treated 3 liter culture after 24 hours, they are inadequate for predicting the future of the population left behind to experience a longer exposure. Thus, a high A.V. of 0.70 for pairs exposed to 1-naphthol is inapplicable to the performance of adults that died without leaving viable cysts in the 3-liter population jar. In contrast, the 1,5-D treated population persisted to 1972 despite a low 1971 A.V. from the pair matings. In this case the moderate proportion of encysted zygotes obtained in the pair mating tests presaged an adequate deposit of viable cysts on the walls of the overwintering population jar. The outstanding example of an impressively persistent population was the one exposed to p-N. Its 1971 A.V. was a low 0.48 but the proportion of zygotes encysted, 33.8%, was higher than the control values.

Following a cycle of evaporation and reconstitution of the overwintering jars, the 1972 A.V.'s for pair matings should adequately represent the performance of the shrimp from the surviving populations. Although none have reached the control level, all show improvement over the 1971 A.V.'s. With Iso, it was possible to show impressive improvement in one generation during the summer of 1972, from an A.V. of 0.26 for the three females and 1 male constituting the first generation to the 0.97 for the pairs used to sample the second generation. Equally important was the increase in the proportion of cysts deposited. Notably the strain

with the lowest A.V. (1,5-D) has responded to stress by producing a high proportion of cysts with good hatchability.

The azinphosmethyl derivatives were gifts from Chemogro Chemical Company, Kansas City, Missouri; the Dursban® derivative from Dow Chemical Company, Midland, Michigan; the carbaryl derivatives from Union Carbide Experiment Station, Clayton, North Carolina; and the diazinon derivative from Geigy Chemical Company, Ardley, New York.

The industrious attention of Janet Guthrie to preliminary experiments must be acknowledged. She was a January plan student from the University of Delaware.

Summary

(1) This paper reports (a) the differential extinction and persistence of 3-liter mass populations following the addition of 10 ppm of 6 derivatives of biodegradable carbamate and organophosphorus pesticides, (b) the reproductive performance of *Artemia* pairs removed from the 6 treated populations after 24 hours, and (c) reproduction tests of pairs from the populations surviving an overwintering evaporation cycle.

(2) Life span was decreased to some degree for adults treated with any of the 6 tested derivatives of "degradable" pesticide. A concomitant decrease in life time totals of offspring can explain the results for 3 of the compounds.

(3) The reproductive performance is further curtailed by cytogenetic destructive action of naphthalene and carbamate types of compounds on gametes and zygotes. The implied mode of action is on dividing cells. Furthermore, related naphthalene compounds are spindle poisons.

(4) An adequate "standing crop" of adults is no assurance of survival of the population. More significant is the presence of either live larvae or viable cysts. The latter are especially important if the culture is subjected to periodic evaporation as is typical of many natural salterns.

(5) Appreciable improvement in the components of reproductive fitness was observed for surviving populations a year after the initial treatment.

LITERATURE CITED

Aly, O. M., and M. A. El-Dib, 1972. Studies of the persistence of some carbamate insecticides in the aquatic environment. *Advan. Chem.*, Series **3**: 210–243.

Carlson, A. R., 1972. Effects of long-term exposures to carbaryl (Sevin) on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*). *J. Fish. Res. Board Can.*, **29**: 583–587.

Collins, T. F. X., W. H. Hansen and H. V. Keeler, 1971. The effect of carbaryl (Sevin) on reproduction of the rat and the gerbil. *Toxicol. Appl. Pharmacol.*, **19**: 202–216.

Frear, D. E. H., and J. E. Boyd, 1967. Use of *Daphnia magna* for the microbioassay of pesticides. I. Development of standardized techniques for rearing *Daphnia* and preparation of dosage-mortality curves for pesticides. *J. Econ. Entomol.*, **60**: 1228–1236.

Fitzhugh, O. G., 1968. Reproductive Tests. Pages 75–85 in N. Boyland and R. Goulding, Eds., *Modern Trends in Toxicology*, Vol. I. Butterworths, London.

Gavaudan, P., and N. Gavaudan, 1940. Action sur la caryocinèse, la cytodiécese, et la morphogénèse des végétaux de quelques dérivés d'hydrocarbures cycliques. *C. R. Soc. Biol. Paris*, **133**: 346–352.

Ghadiri, M., D. A. Greenwood and W. Binns, 1967. Feeding of malathion and carbaryl to laying hens and roosters. *Toxicol. Appl. Pharmacol.,* **10**: 392.

Grosch, D. S., 1965. Biological effects of radiations. *Bios,* **36**: 55–62.

Grosch, D. S., 1966. The reproductive capacity of *Artemia* subjected to successive contaminations of radiophosphorus. *Biol. Bull.,* **131**: 261–271.

Grosch, D. S., 1967. Poisoning with DDT: Effect on reproductive performance of *Artemia*. *Science,* **155**: 592–593.

Grosch, D. S., and A. C. Hoffman, 1973. The vulnerability of specific cells in the oogenetic sequence of *Bracon hebetor* Say to some degradation products of carbamate pesticides. *Environmental Entomology,* in press.

Karinen, J. F., J. G. Lamberton, N. E. Stewart and L. C. Terriere, 1967. Persistence of carbaryl in the marine estuarine environment. Chemical and biological stability in aquarium systems. *J. Agr. Food Chem.,* **15**: 148–156.

Levan, A., and G. Ostergren, 1943. The mechanism of c-mitotic action. Observations on the naphthalene series. *Hereditas,* **29**: 381–443.

Lochhead, J. H., 1941. *Artemia,* the "brine shrimp." *Turtox News,* **19**: 41–45.

Menzie, C. M., 1969. *Metabolism of Pesticides.* U. S. Department of Interior, Bureau of Sport Fisheries and Wildlife, Special Scientific Report Number 127, Washington, D. C.

Mettler, L. E., and T. G. Gregg, 1969. *Population Genetics and Evolution.* Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 212 pp.

Moeschlin, S., 1965. *Poisoning, Diagnosis and Treatment.* Grune and Stratton, New York, 707 pp.

Parke, D. V., 1968. *The Biochemistry of Foreign Compounds.* Pergamon Press, Oxford, England, 269 pp.

Parker, B. L., 1965. A carbamate bioassay technique and improved rearing procedure for *Daphnia magna. Diss. Abstr.,* **26**: 2944.

Pimentel, D., 1971. *Ecological Effects of Pesticides on Non-Target Species.* Office of Science and Technology, U. S. Gov. Printing Office, Washington, D. C., 220 pp.

Robens, J. F., 1969. Teratologic studies of carbaryl, diazinon, norea, disulfiram and thiram in small laboratory animals. *Toxicol. Appl. Pharmacol.,* **15**: 152–163.

Stewart, N. E., R. E. Millemann and W. P. Breese, 1967. Acute toxicity of the insecticide Sevin® and its hydrolytic product 1-naphthol to some marine organisms. *Amer. Fish. Soc. Trans.,* **96**: 25–30.

Strickberger, M. W., 1968. *Genetics.* Macmillan Company, New York, 868 pp.

Weil, C. S., M. D. Woodside, C. P. Carpenter and H. F. Smyth, Jr., 1972. Current status of tests of carbaryl for reproductive and teratogenic effect. *Toxicol. Appl. Pharmacol.,* **21**: 390–404.